

MO-4890

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in an atmosphere of CO₂:O₂N₂ (8:8:84). Supernat was removed and the cells were treated with 0.2% KCL for 5 min. and 0.1% KCL for 25 min.

The KCL was removed and the cells were harvested by scraping. The harvested cells were passed through a 22 gauge needle to break down the cell structure. The cell lysate was subjected to low speed centrifugation for 10 min. and the semi-purified organisms remaining in the supernatant were harvested by high speed centrifugation. Antigen was pooled from 25 flasks and a portion of the antigen was subjected to a french press treatment for the production of soluble antigen. The

Reminder was aliquoted and stored at -70°C. This soluble antigen was formulated into a vaccine according to the following procedure. Vaccine antigen was formulated with TITERMAX® adjuvant or Freunds Incomplete adjuvant at a concentration of 500ug of antigen/dose. With the TITERMAX® adjuvant, 0.5mL was mixed with 0.5mL of antigen to produce a 1.0mL dose containing 500ug of antigen. With the Freunds Incomplete adjuvant, 2.0 mL of adjuvant was mixed with 2.0 mL of antigen such that the total dose also contained 500ug.

A

In order to determine whether the antigen produced could protect pigs from a homologous challenge or from exposure to heterologous isolates or strains, ten 4-week-old pigs were vaccinated and later challenged. Ten control pigs received equal doses of a mock vaccine which contained only the tissue culture medium Minimal Essential Medium (MEM) and adjuvant (without antigen). The vaccine used for the first vaccination contained TITERMAX® adjuvant while the vaccine used for the second vaccination contained Freunds Incomplete adjuvant. Serum samples were taken prior to vaccination (prebleed), at day of booster (Day 14) and at the day of challenge (Day 35) to demonstrate the production of an immune response post vaccination. Serum was tested for antibody to *L. intracellularis* via an ELISA wherein the wells in a 96-well plate were coated with *L. intracellularis* antigen (purified from pig gut epithelial cells) of a clinical isolate which was from a different source than the isolate used to produce the vaccine. Therefore, presence of an